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Molecular diagnosis of *Streptomyces* genus and bioactive potential against pathogenic microbes

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Abstract: This study (40) locally isolated the genus *Streptomyces* from soil samples collected from different regions of Iraq (Nineveh , Erbil , Duhok) and evaluated their antagonistic. The isolates were found to have bioactivity against gram-positive and negative bacteria and fungus. *Streptomyces* were isolated on (S.G. medium), and morphological similarities and the 16 srRNA sequencing were used to characterize them. The results of a polymerase chain reaction (PCR) with eight strands of DNA gene picked from local bacteria isolates in a volume range of (900–1000) base pairs. The nitrogenic base sequence determined the polymerase chain reaction products of DNA samples selected from 6 local isolates. These strands preserved the employed DNA ladder volume. According to DNA Blast NCBI data, the species are Streptomyces atrovirens, Streptomyces SP.S. coeuleroubidus, and Streptomyces bellus.

Key words: *Streptomyces*, Molecular, Pathogenic Microbes.

Introduction

Among the microbe, actinomycetes are one of the critical sources for the production of antibiotics. About two–Streptomyces alone[1] produce thirds of the antibiotics. *Streptomyces* are filamentous bacteria gram a positive with a high (G+C) content (70%) and found in all environmental[2].

The genome code includes more than 20 genetic groups for secondary metabolites with high medicinal potential, such as antibiotics, which help fight against microbes[3].

Many studies have found that Streptomyces has a considerable potential for producing secondary products such as antibiotics, growth factors, and pesticides[4]. Streptomyces can be distinguished from other actinobacteria by their 16 srDNA analysis and DNA - DNA hybridization[5].

Materials and methods

Collecting of samples

(20) Soil samples were gathered from various farms in Nineveh at depths ranging from 5 to 10 cm below the earth's surface. Calcium carbonate (CaCO3) treated the samples at a 1:10 ratio. They were then dried for 4 days at (40–45) degrees centigrade. Samples are collected in plastic bags, closed tightly, and then transferred to a Freezing container until needed[6].

Isolation of Streptomyces

(1) gram of dirt was well mixed in tubes with 10 ml of distilled water; then, a series of dilutions were performed. produced until the desired concentration was reached.10^{-6}, 1 ml was taken from the last dilutions (10^{-5} - 10^{-6}) and placed on culture medium Starch – casein medium (S.G.)

This was done three times for each sample (chilled to 45 degrees centigrade). The inoculum was then disseminated evenly using a sterile glass spreader and incubated for 7 days at 28 C0. Plates with 10–35 colonies were chosen, and the number of solitary colonies was re-cultured in the same medium to generate pure culture[7,8].

The media

Starch – casein medium

It was made by using the following ingredients: (18) gm Agar (10) gm starch, (0.3) gm casein, (2) gm KNO3, (2) gm NaCl, (0.02) gm CaCO3, (2) gm KH2 PO4, (0.05) gm MgSO4 7H2 O (0.01) gram FeSO4. 7H2O, in (1) liter distilled water with a pH of (7.2). After that, the Autoclave apparatus was used to sterilize them[9].

Nutrient agar medium

By melting 23 grams of the medium in (1) liter of distilled water with a pH of (7.2) and sterilizing everything in the autoclave, this medium was created according to (oxoid) the supply firm's recommendations.

Starch mineral salts media

The following ingredients were used to make it: (10) gm Starch, (2) gm (NH4)2 SO4, and (2) gm CaCO3. (1) gram of K2 HPO4, (1) gram NaCl, (20) gm Agar in distilled water with a pH of (7.0), and they were all autoclave sterilized[10].

2.3.4 Glycerol asparagine agar medium

Prepare this medium by dissolving: (1) gm asparagine, (10) gm glycerol, (1) gm K2HPO4, (20) gm agar, (1) ml of trace salt solution, (0.64) gm CuSO4. 5H2O. (0.11) gm FeSO4 . 7H2O, (0.79) gm MnCl2. 4H2O, (0.15) gm

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ZnSO4. 7H2O, The materials were dissolved in (1) liter of distilled water at pH (7.4) and sterilized in an autoclave[11].

Antibiotics Production Medium

The medium for enhanced antibiotic synthesis was created by mixing (0.8) gm NaCl, (1) gm NH4Cl, (0.1) gm K2HPO4, (0.2) gm MgSO4 7H2O, (0.1) gm CaCl2, (10) gm glucose, and (3) gm yeast extract. The materials were dissolved in (1) liter of distilled water at pH (7.3) and sterilized in an autoclave[12].

Isolation and characterization of Streptomyces

The Gram staining, colony morphology, and color of colonies, the starch mineral agar media, and using the slide culture technique been noticed the shape of the aerial and substrate mycelium, as well as the spore arrangement were used to characterize *Streptomyces*[12].

Assay the antagonistic activity of isolates in this experiment

Staphloccocus aureus Proteus vulgris, E. coli, Klebsilla, Pseudomonas aeruginosa (and pathogenic fungi (Candida albicans, Rhizoctonia solani, Fusarium solani, Alternaria alternata .

Antifungal activity against phytopathogenic fungi

To evaluate the effect of *Streptomyces* isolates on the growth mycelium of plant pathogenic fungi, the *Streptomyces* isolates were transferred to the middle of a petri dish, distributed vertically in the petri dish by a sterile loop and incubated at 28-+1 ^{0}C for (5) days, after the incubation period two discs from the eight-day-old fungal culture were transferred to half dishes (1.5) cm away from the growth line of the *Streptomyces* isolates. The control treatment contains fungi discs only, which are incubated 28-+ 1 for 5 days (the incubation period depends on the arrival of the mycelium fungi to the edges of the dish[13].

Antibacterial activity against bacteria pathogen

The bioactivity of the *Streptomyces* was studied against the microbial Agar diffusion method[14]. Asparagine glycerol agar plates were inoculated with Streptomyces isolates and incubated at 28-+ 1 ^{0}C FOR (7) days. After the isolates were grown, 8 (mm) discs were taken by a sterile cork borer from the developing colonies of *Streptomyces* and transferred to the nutrient agar medium inoculation with pathogen bacteria.

Diagnosis of selected samples of bacteria *Streptomyces*

The selected Streptomyces was diagnosed to the species level using the diagnostic tests[12,15-17].

Preparation of inoculation

The elements in the inoculation medium are the same as those in the production media. They were cultivated in autoclave-sterilized 250 mL flasks with 50 mL each. They were inoculated by transferring a tip of Streptomyces cultured on Glycerol asparagine agar to the inoculum. The flask was then shaken in a shaker incubator for 3 days at 140 rounds per second at (28-+1) Celsius.

Cultural Condition

In a 250 mL flask, 50 mL of antibiotic manufacturing media was created. The flasks were tightly sealed, and sterilization in the autoclave was conducted. They were all allowed to cool before injecting inoculum prepared from the 3-day-old chosen isolation at a 2:1 (v/v) ratio. The flaks were kept in an incubator vibrator for 7 days at (28+- 1) degrees Celsius and 240 round minutes.

Purification and acquisition of DNA from *Streptomyces*

The DNA from the *Streptomyces* samples was extracted using Geneaid's analysis kit.

PCR reactions: Genomic DNA Extraction

The T.E. (the solution was used to optimize the DNA concentration in all of the samples used in this experiment, and it was successful in obtaining the desired concentration for the PCR reaction to continue (50 nanograms per microliter).

In a (0.2 ml) Eppendorf tube provided by the British business, the DNA sample was mixed with the specific primer for each gene to make the master reaction for each PCR reaction (bio labs). Using distilled water, the reaction volume was set to 20 microliters, and the reaction was placed in a microfuge for 3–5 seconds. The tubes were then placed within a heat cycler, which used a customized program for each reaction to achieve the polymer reactions. The samples were then electrophoresed using a bio labs ladder in a well of a 2 percent agarose gel for 60–70 minutes, and the gel was filmed using Gel documentation.

Streptomyces molecular diagnostics based on 16srRNA amplification

To identify the amplification area, Add 4 microliters (100 nanograms) of DNA template and 1 microliter (10 picomols) of each gene primer were added to the final mix

Primer Forward: AAGCCC TGG AAACGGGGT

Primer Reverse : CGTGTGCAGCCC AAGACA

After that, the reaction tubes were placed in the thermal cycler, and the polymers reaction was carried out according to the program listed in the table. (1).

DNA Sequencing analysis

DNA sequencing is the most frequently and commonly utilized technique for finding mutations and SNP variants in DNA samples. The sequence of amplified portions of DNA is used to determine, and PCR reactions usually determine research mutations. DNA sequencing results have gotten more precise in determining mutations in recent years[18,19]. If the PCR reaction produces many strands, they are purified, and the desired section of the DNA is recovered from the gel; however, if the reaction produces just one strand, it will be the predominant strand and will be used to identify the sequence[20].

Using DNA sequencing to determine the nucleotides of the amplifier section

The genetic sequences of the samples were determined using the PCR technique outlined previously. Used in the study, and the primers were analyzed with the Hitachi 3130 Genetic Analyzer device. The results were analyzed using the BLAST tool, which compared the genetic sequences to those in the National Center for Biotechnology Information) NCBI(database.

Diagnosis

The Streptomyces samples were identified using the slide culturing technique, one of the best techniques (on a genus level) to display substrate and hyphae, which are the differentiating traits that identify thread-like bacteria[16].

NO.	Stage	Temperature	Time	Cycle number
1	Initial denaturation	95	6 min	1
2	Denaturation	95	1-30 min	35
3	Annealing	58	1-30 min	
4	Extension	72	2 min	
5	Final extension	72	5 min	1

Table 1. Shows the PCR reaction inside the thermocycler device.

The substrate hyphae are spore-free, well-branched, and unsegmented. While the aerial hyphae appeared to be a darker, thicker, and less branched thread than the substrate mycelium, the aerial hyphae have a sporophore containing chain of spores, which can be erect (rectus), spiral (spiral), or Crict with waves (rectus – flexible) depending on how these spores are arranged21. figure (1) .

When the isolates were cultivated on different media types, they revealed a variety of colors. They could not produce melanin and other colors, with gray colonies being the most common[21].

Genomic DNA polymers reaction

Using the forward primer, the following DNA,s reaction was performed on pure DNA acquired from organisms gathered from samples depending to the Geneaid protocol:

(AAGCCCTGGAAACGGGGT) And the revers:(CGTGTG CAGCCCAAGACA). (Maleki et al.,2013) In Figure 3, it can be seen that strands of pure DNA from the samples are of the same length (1000–9000) base pairs generated from the Streptomyces DNA specified polymer process. It has been discovered that they have the same length, indicating that there are nitrogenous base sequences in DNA that are mutual.

These isolates' genomic DNA can link with the primer and continue the reaction, resulting in additional DNA strands of the same length. These results are similar to what was found[22] When utilizing the polymers reaction approach to identify local bacterial samples and acquire a positive result, the length of the DNA created was (1000) nitrogenous base pairs, and so on. When using the polymers reaction method to analyze a bacterial local sample identification, the length of the DNA obtained was 1000 nitrogenous base pairs and so on (μ) marker evaluating and assessing the sequence of nitrogenous bases obtained from particular PCR., from local samples.

The findings of the specific polymer process on isolated DNA from local samples were forwarded to the NCBI, where they were compared to sequences in the gene bank figure (4).

Antifungal activity against phytopathogenic fungi

In this study of the ability of *Streptomyces* isolates to inhibit plant pathogenic fungi Table (2). The isolates *Streptomyces satrovirnes* showed inhibit against fungi (F*usarium solani , Rhizoctonia solani , Alternaria ignifica , Candida albicans*), while the isolates *S. coeuleorubidus*, S. sp, *S. bellus, inhibited fungi C. Albicans, F. solani, and A. alternate* respectively Figure(5).

The *Streptomyces* can be biologically controlled by resistance / or killing fungi and bacteria pathogens; they are often Bioactive before the pathogen thoroughly infects its host. Recently a focus has been placed on different methods of developing biocontrol strategies against soil-borne fungi[23]. For example, the seed of the *Arabidopsis thaliana*.

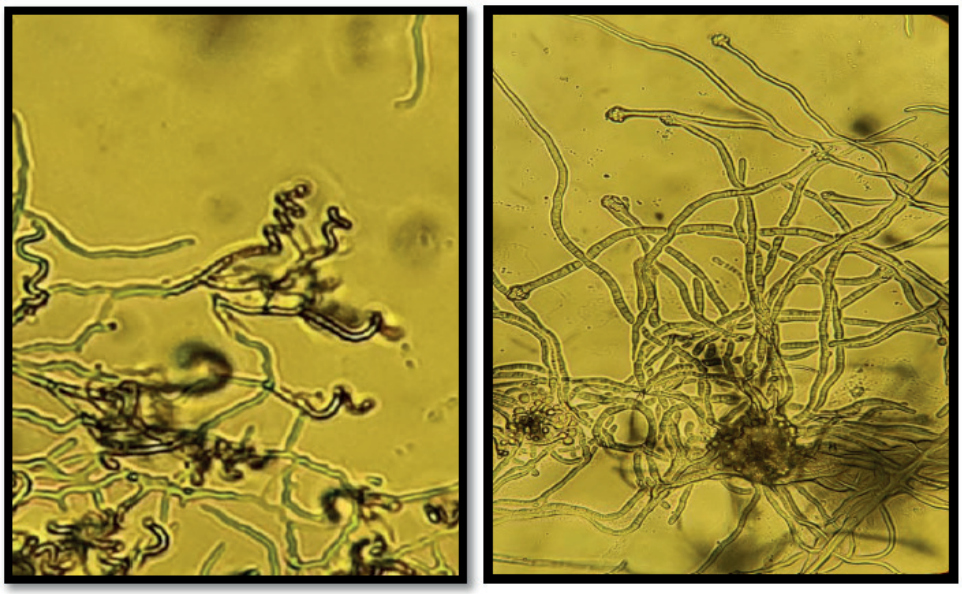

Figure 1. The shape of the substrate and aerial mycelium of *Streptomyces* SPP.

Figure 2. The shape of colonies and pigment production of *Streptomyces*.

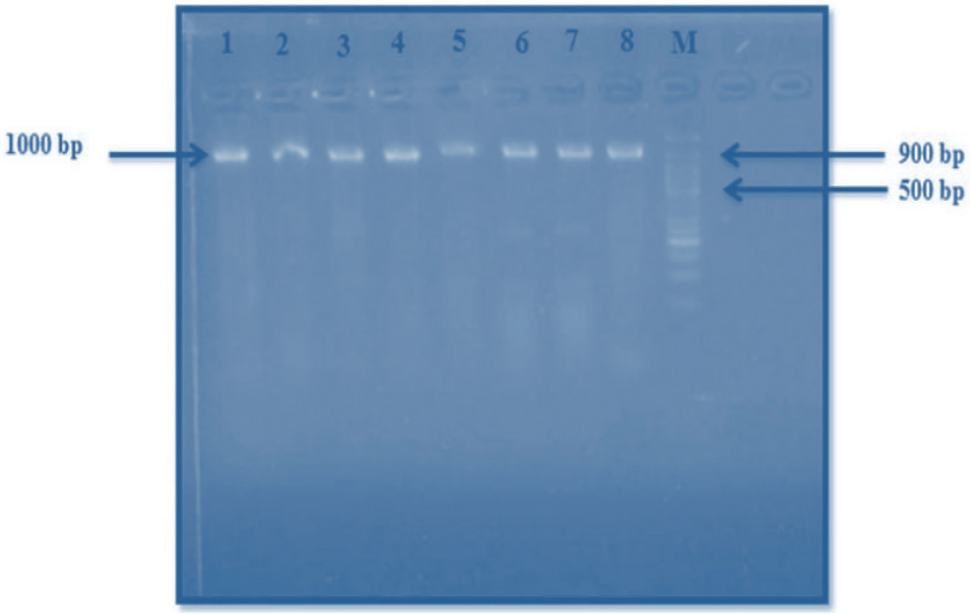

Figure 3. The specific polymerase reaction of *Streptomyces spp.* Primer 16s r RNA.

Before sowing, treatment with Streptomyces and Micomonospora SP was protected from infection by *Erwinina carotovra* and *F. oxysporum*.

It has been observed that *Streptomyces* have an antagonistic ability to stimulate the defense mechanisms in the plant[24].

In this study[25]. Indicated a signification inhibition of mycelium growth in dual culture, using three strains of *Streptomyces*. S.P.ALP 07R, Streptomyces S.P. MRA 1W, against six types of plant pathogenic fungi *Sclerotinia sclerotiorum, Fusarium oxysporm, f. S.P. Lactuca, Rhizoctonio solani Thielvopsis basicola, Phytophthora S.P. Pythium ultimum*. According to (26), one strain of Streptomyces was found among Streptomyces' one hundred seven isolates. *In vitro* and *in vivo*, S.P. SI RO3 reduced the growth of mycelium *Pestalotipsis theae* by a percentage percent 86.15 percent 93.85, respectively.

Antibacterial activity against bacteria pathogen

In this study, the biological activity of *Streptomyces* against some pathogenic bacteria was also evaluated. The *Streptomyces Bellus* give inhibition against all pathogenic bacteria used in this study, while the *Streptomyces* SP. and *S.atrovirence* were effective against all bacteria except Klebisla Penumonia Table (3). The researchers[27]. Showed that the inhibition extracted by acetate was effective against E SBL – producing *E- coli*. Numerous studies have indicated that the metabolites produced by actinomycetes showed high activity against Gram-negative, including *E. coli*[28]. The *Streptomyces* SMO1 gives a high inhibition against bacteria *Staphylococcus aureus* at a concentration (5 ml) when grown on medium AIA at P.H. (7) for 7 days compared with Streptomycin (5 mg) ampicillin (5 mg) also indicated that among 8 isolates, one isolates S MO1 give inhibition against six bacteria strains except for *K. pneumonia* and *S. typhimurium*[29]. However, due to a double membrane barrier and brown transmembrane efflux, Gram-negative bacteria are more resistant to antimicrobial agents than Gram-positive bacteria[28].

Conclusions

Among the results obtained from both aspects, the molecular study proved its effectiveness and usefulness for plant breeders and the building block for the success of any plant breeding program and the shortening of time in determining the appropriate varieties to conduct crosses, improving the characteristics of the yield.

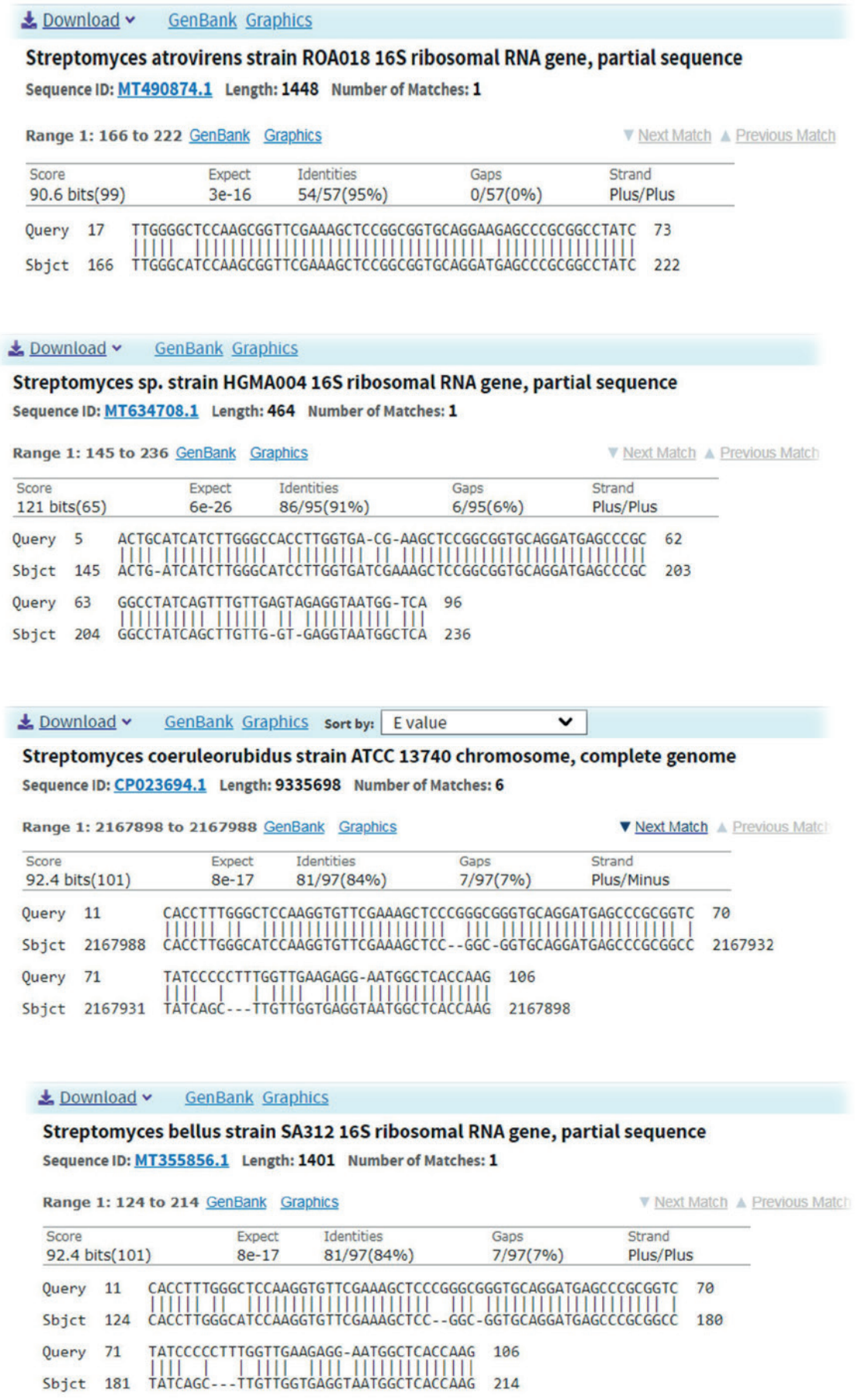

Figure 4. Shows an analysis of the nitrogen base sequence of the gene bank.

Fungi	*Streptomyces SP.*	*S. atrovirens*	*S.Ceouleroubidus*	*S. bellus*
Fusarium Solani	_	14	10	_
Rhizoctonia Solani	_	15	_	_
Alternaria Alternate	_	13	11	8
Candida Albicans	17	14	_	15

Table 2. The effect of *Streptomyces* isolates of phytopathogenic fungi (mm).

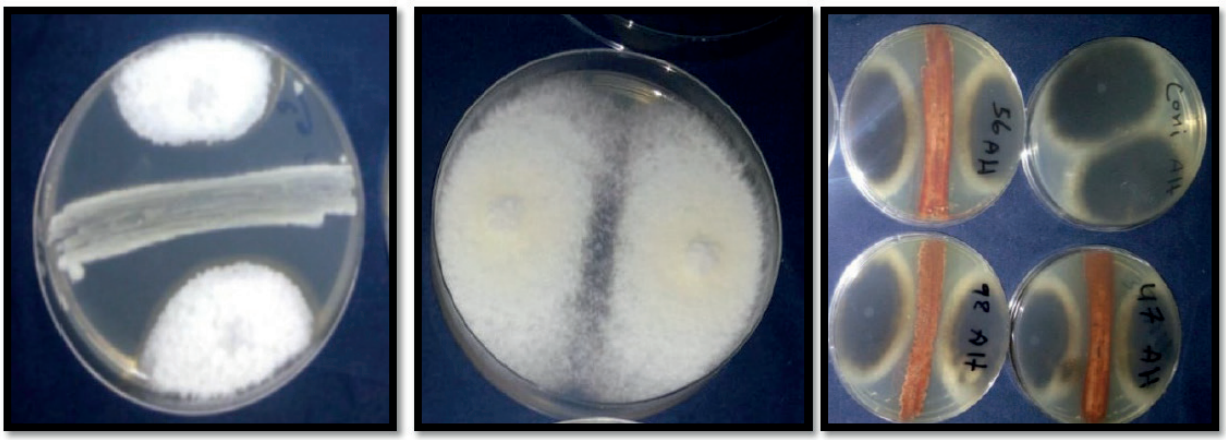

Figure 5. The effect of *Streptomyces* on phytopathogenic fungi.

Bacteria Pathogen	*Streptomyces_SP.*	*S. atrovirens*	*S. Ceauleroubidus*	*S. Bellus*
Staphylococcus Aureus	12	16	_	13
Escherichia Coli	14	11	_	13
Klebsiella Pneumonia	_	_	17	11
Pseudomonas aeuraginosa	14	15	11	14
Proteus Vulgaris	11	12	12	11

Table 3. The effectiveness of *Streptomyces* on bacteria pathogen inhibition zone (mm).

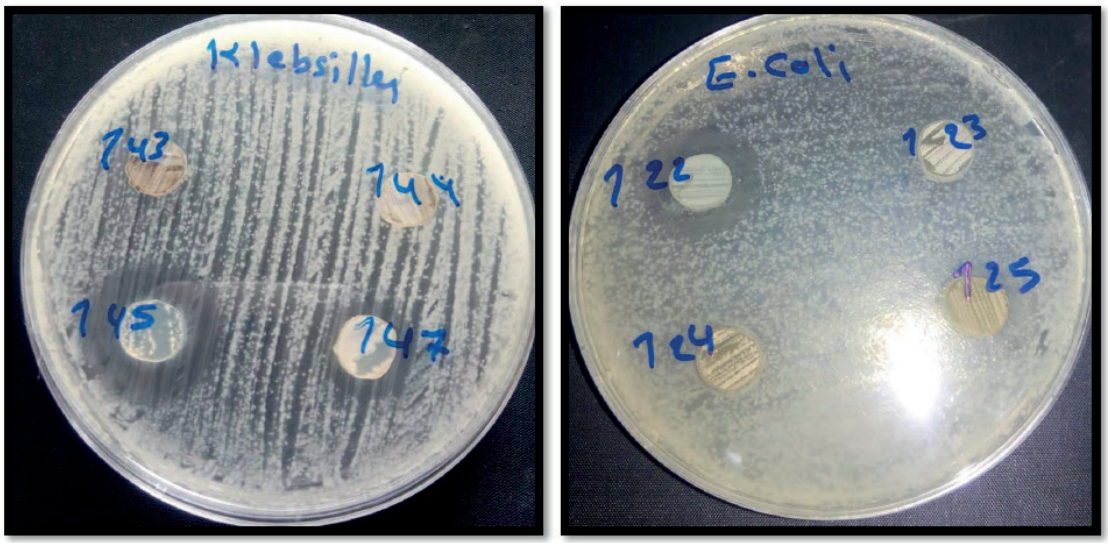

Figure 6. The effect of *Streptomyces* isolates on pathogenic bacteria.

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Conflicts of Interest

There is no conflict.

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